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*CHROMOSOMES OF THE RED FOX**

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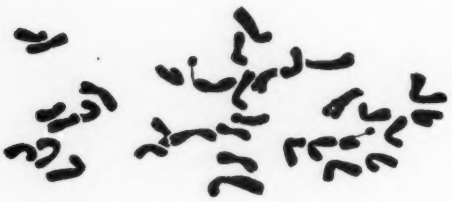
A number of mutant color types have arisen, directly or indirectly, from the red fox (*Vulpes vulpes* L.). Two different mutations to black [standard black (silver) and Alaskan black (silver)] had occurred in wild red foxes of North America prior to ranch breeding. The platinum character is the result of a mutation from ranch-bred standard black (silver) foxes. The present study is concerned with both the number and the comparative forms of the chromosomes in red, standard black (silver) and platinum-silver foxes. Wodsedalek[1] reported 42 as the chromosome number of the male red fox. Later Andres[2] found the number to be 34.

Testes were collected during the breeding season of 1942 in order to obtain meiotic and mitotic divisions. A red and a black (silver) fox were killed by electrocution, and another red fox by an injection of ether into the heart. The material from the platinum-silver fox was obtained by castration. The testes in each case were immediately excised and lacerated, and the seminiferous tubules were teased out and placed in Carnoy's alcohol-acetic acid-chloroform solution (7:2:1). The complete operation required less than five minutes. The tubules were allowed to remain in the fixative from 30 minutes to one hour; they were then transferred to 95 per cent alcohol for an hour and stored in 80 per cent alcohol. Temporary mounts prepared by the aceto-carmine smear method provided excellent material for study.

Polar views of diploid equatorial plates from seminiferous tubules show 34 chromosomes in the red, black (silver) and platinum-silver foxes (Figs. 1, 2, 3). The chromosomes of all three color types appear similar in size, shape and other morphological characteristics. Longitudinal splits and median or sub-terminal spindle fibre attachment regions are common (Fig. 3). The homologues of several chromosome pairs are easily identified. The chromosomes vary in length, the shortest being about one-half that of the longest. A pair of satellite chromosomes is present in each case.



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5

EXPLANATION ON OPPOSITE PAGE

These satellite chromosomes are of medium size and have median spindle-fibre attachment regions (Fig. 3 (*s*)). As a general rule, satellite chromosomes of both plants and animals are found associated with the nucleolus. This may be true in the fox, for a resting nucleus shows chromatic material attached to the nucleolus in two places (Fig. 4).

On the first meiotic equatorial plate the chromosomes are shorter, thicker and more closely packed than in the mitotic divisions; hence the determination of the haploid number is somewhat difficult. However, 17 pairs (Fig. 5), including one pair with attached satellites, are present on the heterotypic equatorial plate. Spindle-fibre attachment regions are easily recognized at this stage. There appears to be more variation in the size of the chromosomes at meiosis than in mitosis.

The occurrence of satellite chromosomes in the fox is of special interest, since, so far as the writers are aware, they have not been previously recorded in mammals. Coonen,[3] in a review of the literature on satellite chromosomes in plants and animals, found them common in plants but comparatively rare in animals. He cites reports of only six animals in which satellites have been noted: the mosquito, *Drosophila*, *Bibio*, *Amblystoma*, *Opalina* and *Salmo*. The present observations agree with those of Andres[2] as to the chromosome number in the red fox, but he makes no reference to satellite chromosomes. His figure 1 shows 34 chromosomes plus a small chromosome for which he gives no explanation. Since Andres[2] used sectioned material instead of smears it may be that the satellite connection was lost or obscured by the method used, and that the extra chromatic particle actually was the satellite.

The platinum-silver character in foxes has been reported[4] as lethal in the homozygous condition. It would be of interest to investigate the possibilities of observable differences in the chromosomes of foxes heterozygous for this character. It is not to be expected that minor differences would be seen at the equatorial plate, but further search may possibly reveal dissimilarities in the pachytene stage.

The writers are indebted to Professor W. Wisnicky, Director of Fur Farm Research, and Professor L. J. Cole for materials and suggestions.

EXPLANATION OF PLATE

Figures 1–3. Equatorial plates from seminiferous tubules of the red fox, figure 1, × 2140; black (silver), figure 2, × 2140; platinum-silver, figure 3, × 2920; showing the diploid chromosome number. Thirty-four chromosomes are present in each case. Figure 3 (*s*), satellite chromosome.

Figure 4. Resting nucleus from seminiferous tubule, red fox, showing chromatic attachments to the nucleole. × 2920.

Figure 5. Equatorial plate, first meiotic division, from seminiferous tubules of black (silver) fox. × 2920.

Further acknowledgment is extended to Professors D. C. Cooper and C. E. Allen for advice and constructive criticisms.

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SALIVARY GLAND TYPE CHROMOSOMES IN MOSQUITOES

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Mosquitoes have been the subject of several cytological studies, but in these insects very little consideration has been given to the type of chromosome characteristic of the salivary glands and other organs of many Dipteran larvae. In recent work, chromosomes from the large larval mid-gut cells of mosquitoes have been described[1,2] as similar to the salivary gland type, but lacking the regular alternation of bands and achromatic regions and consisting merely of a linear series of chromatic masses.

Giant chromosomes with regular banded structure may be obtained, however, from certain tissues in the later stages of development. These chromosomes will be described here. The most satisfactory preparations were obtained from the Malpighian tubes of the imago, pupa or fourth instar larva, but such chromosomes were also found in the salivary glands, gastric caeca and mid-gut of the prepupal stage.

DESCRIPTION OF PLATE

Figure 1. Nucleus from Malpighian tubes of *Culex pipiens* male, pupal stage. Ca. × 510.

Figure 2. Chromosome from *C. pipiens* male, with nucleolus. Ca. × 1160.

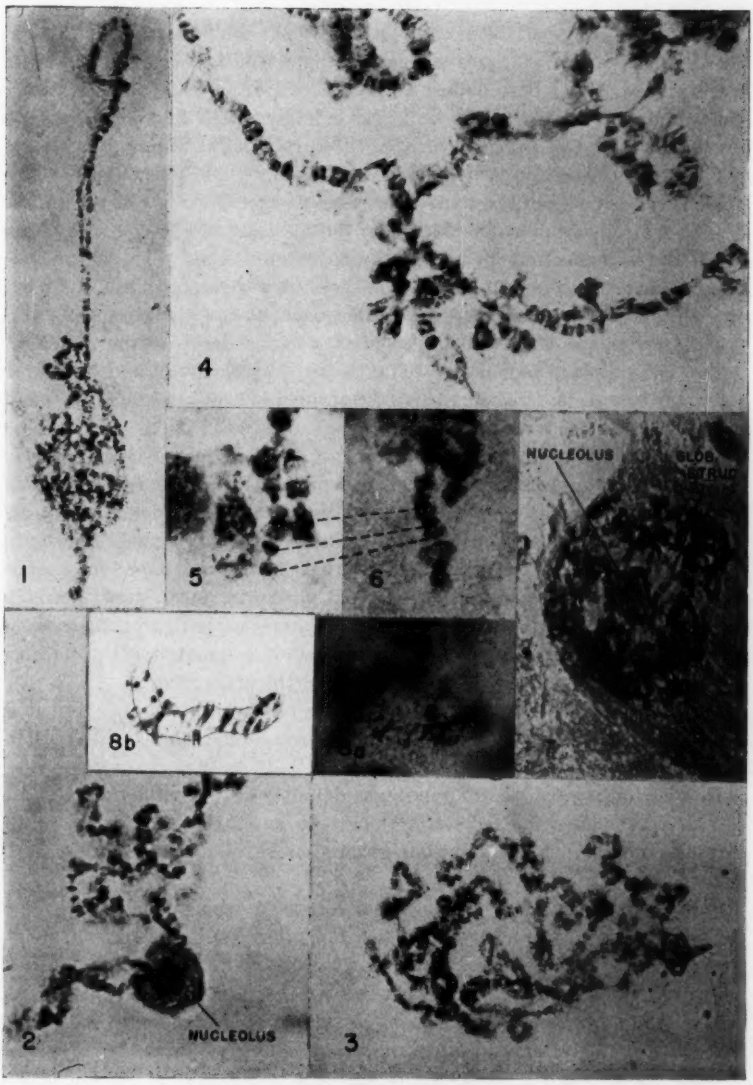
Figure 3. Nucleus from *Aedes aegypti* female. Ca. × 580.

Figure 4. Part of nucleus from *A. aegypti* male showing banded regions and "weak spots." Ca. × 1160.

Figures 5 and 6. Comparison of the same region in two different cells of *A. aegypti*, fourth instar larva. Ca. × 1160.

Figure 7. Nucleus from *A. aegypti*, fourth instar larva, showing globular structure and nucleolus. Ca. × 580.

Figure 8 (*a*). Section of a chromosome from *A. aegypti* pupa, with peculiar banded structure. Ca. × 1160. (*b*) Camera lucida drawing of same.



DESCRIPTION ON OPPOSITE PAGE

In the salivary glands of the adult mosquito the chromosomes are apparently of the normal somatic type.

Technique.—Eggs of the species *Culex pipiens* and *Aedes aegypti*[3] were raised in tap-water at room temperature and the larvae were fed with breadcrumbs. The best slides were obtained from individuals (fourth instar larvae, pupae or newly emerged adults) which had been kept at low temperature (10–18°C.) for two or three days before killing.

The standard technique for smearing salivary gland chromosomes (by staining for a few minutes in aceto-carmine or acetic orcein placing on the slide in a drop of stain and flattening by pressure on the coverslip) was found to be ineffective for the mosquito chromosomes. These chromosomes are very soft, and instead of spreading them out, the standard procedure merely squashes the whole nucleus.

The best treatment that I have found so far is the following modification of the standard technique: The tissue is dissected out and placed for one minute in acetic alcohol. It is then stained in acetic orcein (1% orcein in 45% acetic acid) for about one hour, and smeared in the usual way.

The Salivary Type Chromosomes.—(1) *Culex pipiens.* Figure 1 in the plate shows a whole nucleus from a smear of the Malpighian tubes of *Culex pipiens.* One chromosome pair lies free, and the banded structure can be clearly seen. In some preparations I have seen the three pairs of chromosomes ($2n = 6$), synapsed along most of their length, and completely separated from each other, with no apparent chromocenter. Regions which appear heterochromatic like the chromocenter regions in *Drosophila melanogaster* have not been observed. A nucleolus is associated with one pair of chromosomes near the distal end of one of the arms (Fig. 2).

No detailed study of the banding pattern has been attempted, but it is clear that a map could be made for each chromosome, similar to the salivary gland chromosome maps of Drosophila.

In a cursory study, no difference was detected between the chromosome complexes of males and females. It may be noted that the sexes are not certainly distinguishable by their chromosomes in normal mitotic divisions.[4]

(2) *Aedes aegypti.* Figures 3 and 4 show a whole nucleus of *Aedes aegypti* and part of a nucleus at a higher magnification. The banded structure is clear in some places, but the pattern of banding cannot be followed along individual chromosome arms, because it is interrupted by "weak spots." At these places the chromosomes often fragment in smearing, and parts of the same or of different chromosomes adhere in these regions, so that the continuity of any particular arm is obscured. These contact points can be seen in figure 4. Parts of chromosomes are very often connected by attenuated threads.

As in *Culex pipiens* there is no single chromocenter such as is found in

salivary gland nuclei of Drosophila. It is possible that the contacts are due to non-specific pairing between heterochromatic segments which are interspersed between the banded regions all along the chromosome arms.

Figures 5 and 6 show comparable banded fragments from different nuclei. By a series of such comparisons it would be possible to map the banding pattern for short regions, but it is doubtful whether the sequence for a whole chromosome could be worked out.

Although a nucleolus is present earlier in the development of the nuclei (Fig. 7), it is not visible at later stages after treatment with acetic orcein, in marked contrast to the nucleolus of *Culex pipiens*.

Development of the Salivary Gland Type Chromosomes.—The development of the fully banded chromosomes in mosquitoes has not been studied to any considerable extent, but a few observations, made in the course of dissecting tissues from individuals at various stages of development, may be of some interest. In this respect the two species studied are similar.

The regular banding is not present in chromosomes at the earlier stages (up to third instar larvae), nor do all chromosomes in the Malpighian tubes of later stages necessarily attain this type. In young larvae all, and in later stages some of the cells in question have chromosomes in which the nucleic acid is accumulated in more or less spherical masses of different sizes, separated by achromatic regions (Fig. 7).

Sometimes the globules appear to be connected in a linear series only by fine chromatic threads. In a few figures, short sections of chromosome showed a peculiar banded structure, in which the bands appeared to be connected by paired threads (Fig. 8).

It seems that the dense accumulations of nucleic acid in the globule stage are redistributed (perhaps spread out laterally, or perhaps partially lost) in the formation of the banded type of chromosome. Some intermediate cells are found in the later stages, where well-defined bands are seen in some sections of the chromosomes, while other sections approximate to the globule type.

Tentatively, a comparison may be drawn between this shift in nucleic acid attachment and the experimental results obtained by Kodani.[5] The globule stage described here bears some resemblance to the γ 1 stage in his treated chromosomes. It must be remembered, however, that while the transition in mosquito is a normal developmental process, the apparent reversal of this process obtained by Kodani is induced by somewhat drastic treatment, and it is not yet known whether this induced effect is reversible.

Summary.—A brief description is presented of the development and characteristics of salivary gland type chromosomes in two mosquito species (*Culex pipiens* and *Aedes aegypti*) in both of which the diploid chromosome number is six. The differences observed between the two species indicate

that these chromosomes might be useful as a diagnostic character in distinguishing different species of mosquito.

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PURINES AS GROWTH REQUIREMENTS OF SPIRILLUM SERPENS

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The recognition of the purine and pyrimidine bases as factors affecting the growth of microörganisms has been reported with increasing frequency during the past few years. Richardson[1] in 1936 found that under certain conditions uracil was essential for the growth of *Staphylococcus aureus*. Möller[2] showed that adenine was required for the growth of *Streptobacterium plantarum*, while Pappenheimer and Hottle[3] found adenine to be necessary for the growth of a strain of group A hemolytic streptococci. In the latter case adenine could be replaced by hypoxanthine, guanine, xanthine, guanylic acid or adenylic acid. Furthermore, it was observed that purines were not required by this organism if the carbon dioxide tension above the medium was maintained sufficiently high. Snell and Mitchell[4] have reported the requirements of several lactic acid bacteria for purine and pyrimidine bases. *Streptococcus lactis* was found to require adenine and thymine for growth. Guanine was found to be essential for the growth of *Leuconostoc mesenteroides*, while uracil stimulated the growth of the latter organism and of *Lactobacillus arabinosus*. Robbins and Kavanagh[5,6] have recently described the effect of guanine and hypoxanthine on the growth of *Phycomyces*. In the present communication we wish to report the essential nature of several purine bases for the growth of a strain of *Spirillum serpens*.

Investigation of the cultural requirements of this organism (carried by American Type Culture Collection as No. 8084) showed that although it could not be grown in simple synthetic media the addition to such media of small amounts of natural extracts caused growth to occur. The active principle of such extracts was isolated and was found to consist of purine bases. Hypoxanthine, adenine and guanine were found to affect the growth of the organism.

Experimental.—The basal medium used had the following composition:

	AMOUNT PER LITER OF MEDIUM
Asparagine	5.0 g.
Inorganic salts:	
KH_2PO_4	500 mg.
K_2HPO_4	500 mg.
$MgSO_4 \cdot 7H_2O$	200 mg.
NaCl	10 mg.
$FeSO_4 \cdot 7H_2O$	10 mg.
$MnSO_4 \cdot 4H_2O$	10 mg.

The pH of the medium was adjusted to 7.2. The addition of the proper purine bases to this medium completely met all requirements of the organism. None of the "B-vitamins" was required.

Cultures were grown in 50 ml. Erlenmeyer flasks. Solutions of materials to be tested were pipetted into the flasks and the volume in each was adjusted to 5.0 ml. with distilled water. To each flask was then added 22.0 ml. of the basal medium described above (27 ml. cultures were required to fill the absorption cell used in determining extent of growth). The flasks were plugged with cotton, sterilized by steaming for 15 minutes, cooled and inoculated. Inoculum was prepared from 24 hour cultures of the organism in beef extract-peptone broth. The cells of such a culture were centrifuged out aseptically, resuspended in sterile water, and one drop of the resulting suspension was used to inoculate each flask.

The growth of the organism was measured by quantitatively comparing turbidities in a thermoelectric turbidimeter.[7] Growth was expressed directly as galvanometer readings.

TABLE 1

HYPOXANTHINE AND GUANINE

(Growth Expressed as Turbidimeter Galvanometer Readings)

μG. HYPOXANTHINE PER 27 ML. CULTURE	μG. GUANINE ADDED PER 27 ML. CULTURE		
	0	10	200
0	29.7*	....	..
3	38.2	30.5	28.2
6	48.5	37.8	29.0
12	59.9	60.0	30.0
30	64.0	64.0	33.0
100	65.8	..	..
250	65.8	..	65.0

* Absorption cell filled with distilled water gave a reading of approximately 25.

The effect of the addition of hypoxanthine to the basal medium is shown in table 1. This compound alone is able to replace all of the growth-promoting activity of natural extracts. In the third and fourth columns of table 1 is shown the effect of the addition of guanine to cultures containing

hypoxanthine. Guanine inhibits the growth stimulated by hypoxanthine, provided the concentration of guanine is greater than that of hypoxanthine. If the amount of hypoxanthine present is in excess of the amount of guanine, the latter factor has no effect on growth.

TABLE 2

HYPOXANTHINE AND ADENINE

(Growth Expressed as Turbidimeter Galvanometer Readings)

μG. HYPOXANTHINE PER 27 ML. CULTURE	μG. ADENINE ADDED PER 27 ML. CULTURE						
	0	2	5	10	50	100	200
0	28.0	..	..	..	..	..	..
2	33.5	38.3	35.1	29.8	..	..	..
5	41.0	45.8	47.8	39.1	28.0	28.0	..
10	50.5	52.2	54.8	52.5	29.5	28.0	..
15	55.8	..	..	..	..	..	..
100	67.0	..	..	67.0	66.0	51.0	33.8

The effect of the addition of adenine to cultures containing hypoxanthine is shown in table 2. It can be seen that if the concentration of adenine is equal to or less than the concentration of hypoxanthine, growth greater than that produced by the hypoxanthine alone occurs. If the adenine concentration is greater than that of hypoxanthine, toxic effects are observed, and if the ratio of adenine to hypoxanthine becomes large, the physiological activity of the latter compound is entirely masked. It should be noted that when the concentrations of both factors are large, the toxic effect of adenine is apparent at lower adenine-hypoxanthine ratios than at lower concentrations.

Uric acid, xanthine and uracil have no effect on the physiological activity of hypoxanthine.

TABLE 3

PHYSIOLOGICAL ACTIVITY OF ADENINE AND GUANINE

(Growth Expressed in Turbidimeter Galvanometer Readings)

μG. GUANINE PER 27 ML. CULTURE	μG. ADENINE PER 27 ML. CULTURE						
	0	5	10	20	50	100	250
0	27.0	32.5	31.1	30.0	29.5	29.0	28.7
5	25.9	55.0	56.1	55.0	34.8	30.8	29.4
10	25.8	46.5	58.0	60.5	55.0	34.8	30.0
20	26.0	32.9	56.0	63.0	60.0	53.0	31.0
50	25.8	30.0	36.8	56.7	63.5	60.0	44.1
100	26.0	30.1	31.8	45.0	57.5	54.2	44.3
250	26.0	28.9	30.1	37.9	55.0	53.5	51.0

Table 3 shows the effect of the addition of various combinations of adenine and guanine to the basal medium. Neither adenine nor guanine alone is

active, but a mixture of approximately equal parts of the two is able to supply the requirements of the organism. If either component of the mixture is appreciably in excess of the other, toxic effects are observed, and if the imbalance is extreme, the physiological activity is completely masked. Neither the purine, xanthine nor the pyrimidines, uracil, cytosine or thymine, have any effect on the action of adenine or guanine. Adenosine and yeast adenylic acid cannot take the place of adenine.

Discussion.—The growth requirements of *Spirillum serpens* are completely met in a medium composed of asparagine and inorganic salts supplemented with hypoxanthine or an equimolecular mixture of adenine and guanine. The organism thus differs from other organisms which have been reported to require purines in that all of the latter organisms have rather complex requirements in addition to the purines.

The reversible inhibitory action of certain purines on the physiological activity of others has been described. A similar relationship has not hitherto been reported in the case of the purine requirements of microörganisms, but several examples of a similar phenomenon with other nutrilites have been reported. McIlwain[8] showed that pyridine-3-sulfonic acid or its amide interfered with the growth of organisms requiring nicotinic acid. *p*-Aminobenzoic acid in small amounts has been reported to overcome the bactericidal effects of sulfanilamide and its derivatives.[9] Snell[10] has reported that the sulfonic acid analogue of pantothenic acid inhibited the growth of organisms which require this vitamin. The addition of excess pantothenic acid reversed the inhibition.

The toxic effects of the pyridine-3-sulfonic acid, the sulfanilamide compounds and the pantoyl taurine are believed due to the fact that they are structurally very similar to the corresponding naturally occurring nutrilites. The inhibitory substances thus appear to be able to fit into the biochemical patterns normally occupied by the essential nutrilites. They are not, however, able to carry out whatever vital functions are performed by these compounds and the metabolic processes of the organism are blocked at this point.

This picture can be applied in the present instance, but is complicated by the fact that adenine and guanine, which are inhibitory under certain conditions, contribute to the growth of the organism under other conditions. In seeking to rationalize these results the following speculation is suggested.

Assume that the purines function in a physiologically essential complex that contains two purine bases per molecule. (Several important coenzymes are known to have a dinucleotide structure.) Then, according to the results obtained, this complex can function only if (1) both purines are hypoxanthine, (2) one purine is hypoxanthine and the other adenine or (3) one purine is adenine and the other guanine. The complex cannot function physiologically if (1) both purines are adenine, (2) both purines are guanine

or (3) one purine is hypoxanthine and the other guanine. Xanthine and uric acid do not compete for places in the complex. Such an hypothesis is in agreement with all of the observations, and is useful in indicating possible answers to a number of questions. For example, on this assumption it can be seen how it is possible for an excess of adenine or guanine to be toxic while an equimolecular mixture of the two promotes growth, and how it is possible for an excess of adenine to inhibit the action of hypoxanthine while a lower concentration adds to growth.

In line with the above hypothesis it is interesting to recall that Stockstad[11] found *Lactobacillus casei* to require what appeared to be a dinucleotide isolated from liver. This dinucleotide could be partially replaced by a mixture of purine and pyrimidine bases, but only by amounts much greater than required of the dinucleotide.

Summary.—Hypoxanthine or an equimolecular mixture of adenine and guanine is essential for the growth of *Spirillum serpens*. Adenine or guanine alone does not support growth, and under certain conditions these compounds are toxic to the organism. No other vitamin-like compounds affect the growth of the organism.

An hypothesis is suggested concerning the toxic effects of adenine and guanine.

The author wishes to thank Dr. R. J. Williams for his advice and encouragement during the course of this work.

* Standard Brands Fellow for 1941–1942.

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THE DISTRIBUTION OF METALLIC ATOMS IN TWO-COMPONENT GLASSES

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1. Introduction.—Most of the present knowledge of glass structure is based on the work of Warren and his school in the interpretation of x-ray diffraction patterns. In the method used by Warren, a diffraction pattern is converted into an intensity curve from which, by application of the Fourier integral, a radial distribution curve is computed. This curve gives an indication of the amount of scattering matter at any distance from any atom. Thus, in the radial distribution curve of silicon dioxide glass, at the shortest interatomic distance, silicon to oxygen = 1.62 Å, there is a well-defined peak. The area under this peak represents four oxygen atoms, an indication that the tetrahedral arrangement of oxygen atoms about silicon which is found in all crystalline silicates obtains in glass. At the next shortest interatomic distance, oxygen to oxygen = 2.65 Å, there is another peak, poorly resolved, but which may be considered to represent approximately six oxygen atoms about each oxygen atom. A third peak occurs at twice the silicon to oxygen separation and may be interpreted as representing the silicon to silicon distance. At larger radial distances, there is so much overlapping of peaks due to the great number of interatomic distances and to the fact that the peaks become broadened because of the random nature of glass structure that further interpretation is difficult.

Similar curves have been computed by Warren and his co-workers for series of glasses consisting of soda-silica,[1] soda-boric oxide,[2] potassium oxide-boric oxide[3] and pure silica and pure boric oxide.[4] In all of these cases only the shortest two or three interatomic distances can be interpreted. No information can be obtained regarding the distribution of the metallic atoms.

2. The Differential Method.—Figure 1 (*a*) shows a typical radial distribution curve for soda-silica glass as obtained by Warren and Biscoe.[1] The peak due to the silicon to oxygen separation, 1.62 Å, is clearly resolved. A second peak occurs at a radial distance of about 2.4 Å. This is poorly resolved and is, actually, two peaks, one due to sodium to oxygen at 2.35 Å and one due to oxygen to oxygen at 2.65 Å. Figure 1 (*b*) is the curve for pure silica glass, also as obtained by Warren and Biscoe. It occurred to the writer that subtraction of the ordinates of the curve of figure 1 (*b*) from those of the curve of figure 1 (*a*) would eliminate all peaks common to both curves and leave only those due to the radial distribution of scattering

matter about sodium, thus providing information about the distribution of the metallic atoms. If this is done, a differential curve, figure 1 (*c*),

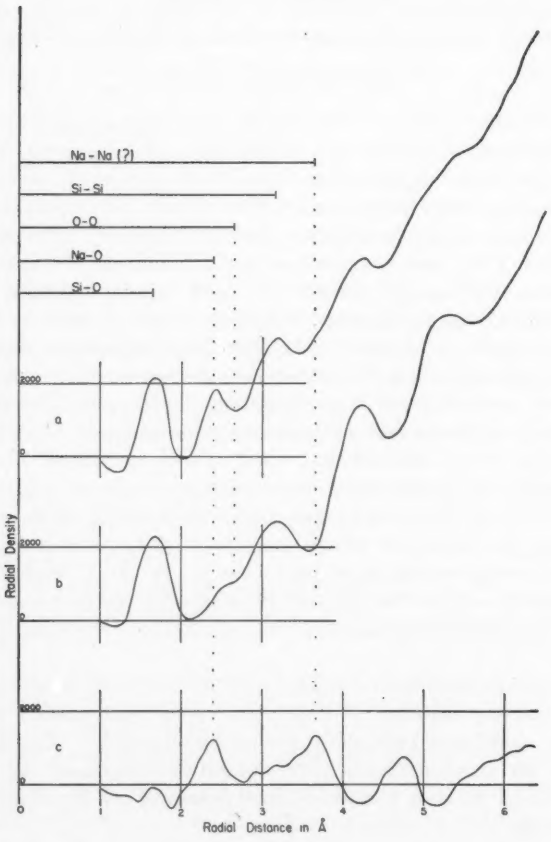

FIGURE 1

(*a*) Radial distribution curve of 19.5% Na_2O·80.5% SiO_2 glass (after Warren and Biscoe).

(*b*) Radial distribution curve of SiO_2 glass (after Warren and Biscoe).

(*c*) Differential radial distribution curve obtained by subtraction of silica glass curve from soda-silica glass curve.

results which shows a series of sharp peaks, most of which were not evident in the radial distribution curve of soda-silica glass. There are sharp,

major peaks at distances from the origin of 2.4, 3.65 and 4.75 Å. A fourth major peak occurs in the region of 6.3 Å, but this is less well resolved. These major peaks persist throughout the entire composition range studied by Warren and Biscoe.[1] There are also a number of minor peaks which may or may not be significant, depending upon the extent to which they are affected by errors inherent in the method.

The first major peak on the differential radial distribution curve is due to the sodium–oxygen interatomic distance of 2.35 Å. This peak was observed by Warren and Biscoe[1] on their curve, figure 1 (*a*), where it appears unresolved from the oxygen-oxygen peak at 2.65 Å. The position of the peak remains substantially unchanged with increasing soda content of the glass, but the area beneath it varies, increasing in general. The second major peak may be attributed to the first sodium–sodium distance. Its position shifts slightly with increasing soda content, and the area beneath it increases regularly, both of which facts are consistent with an interpretation of the peak as being due to a sodium–sodium interatomic distance. The third peak may be due to a second sodium–oxygen distance or a second sodium–sodium distance.

Since the primary purpose of this paper is to outline the principle of the differential method, the above interpretation of the peaks should be considered to be illustrative rather than conclusive. The available data were not designed for this type of treatment, and the results are naturally somewhat crude. It is hoped to supplement this superficial analysis when better data are available.

3. Sources of Error.—There are certain sources of error that affect the nature of the differential curve and complicate its interpretation. The data used to construct the curve in figure 1 (*c*) were taken from published curves which in themselves are accurate only to about one part in ten, and the process of subtraction makes the result subject to considerable error. For this reason, only major peaks which persist throughout the entire composition range can be considered as being significant. Minor peaks may be real, but they may also be due to errors in plotting or measurement.

A theoretical error is introduced by the assumption that the radial distribution of scattering matter about silicon and oxygen does not change. For the radial distances involved here, it seems safe to assume that the interatomic distances do not change appreciably, but the area beneath the curve becomes smaller. In the first place, the area beneath the curve representing the distribution of scattering matter about silicon and oxygen depends upon the total number of silicon and oxygen atoms present in a given volume, and this number decreases as the soda content increases. Also, the number of oxygen atoms about each oxygen becomes less with increasing soda content.[1] The net effect is the subtraction of too much

density. The areas of the residual peaks are too small, and their positions may be shifted slightly.

4. Sodium and Potassium Borate Glasses.—Curves similar to figure 1 (*c*) have been constructed for soda-boric oxide glasses, using the curves of Biscoe and Warren[2] and for potassium oxide-boric oxide glasses, using the curves of Green.[3] The interpretation of these curves is made the more difficult by the fact that the coördination of boron changes from three to four with increasing metal content.[2, 3] The residual peaks are subject to more error than those of silica glasses where the coördination groups of silicon and oxygen do not change. However, in the case of the soda-boric oxide glasses, the differential radial distribution curves show sharp peaks at 3.4 and 4.5 Å which may be due to sodium–sodium distances. In the potassium oxide–boric oxide series, the differential curves show a sharp peak at 3.3 Å which may be the potassium–potassium separation. In both series of glasses, the area under the peaks which are tentatively assigned to the metal to metal separation increases regularly with increasing metal content.

5. Extension and Improvement of the Differential Method.—In an accompanying article,[5] M. J. Buerger discusses the application of the differential technique to the synthesis of Fourier series in crystal structure determinations. Applying the idea of atom for atom substitution developed by him to the glass problem, it is possible to eliminate one of the sources of error, namely, the change in area due to the change in coördination of oxygen atoms about oxygen atoms and to the decrease in absolute numbers of silicon and oxygen atoms present in a given volume. The curve of a lithia-silica glass, for instance, might be subtracted from one of a soda-silica glass of the same molal composition. Assuming the silicon-oxygen framework to be the same in the two glasses, and adjusting for the change in absolute numbers of silicon and oxygen atoms, if necessary, one may entirely eliminate the radial distribution about silicon and oxygen. (Peaks due to the distribution about lithium would be small, and only minor errors would be introduced by subtracting them. These errors might be accounted for by computing the expected areas of the peaks.) One may also extend the differential technique to liquids and solutions, or, in some cases, to gases. In effect, with appropriate variations of composition, radial distribution curves may be computed for the density of scattering matter about any desired atomic species.

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A NEW FOURIER SERIES TECHNIQUE FOR CRYSTAL STRUCTURE DETERMINATION

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1. Introduction.—In an accompanying paper,[1] Joseph S. Lukesh demonstrates that important information can be derived about interatomic distances in glasses provided use is made of data from two related glasses. Specifically, if one prepares a curve by subtracting from the ordinates of the radial distribution curve of a two-component glass the ordinates of the radial distribution curve of one of the two components, there results a differential curve. This new curve has maxima at abscissae corresponding to the interatomic distances of the additional metal atoms in the two-component glass.

When the writer first learned of this discovery, it occurred to him that a corresponding difference function might be set up for two crystals of related structure and that this function would be capable of providing the interatomic vectors of the atom contained in one structure but absent in the other. A more careful examination of the situation, however, proves that an appropriate extension of the difference idea to Patterson diagrams gives rise to a new diagram having rather remarkable properties.

2. Properties of the Patterson Diagram.—Patterson has shown[2,3] that the Fourier summation

$$A_{x,y} = \sum\sum \mid F_{hk0} \mid^2 e^{2\pi i(hx + ky)} \qquad (1)$$

has peaks at points corresponding to the ends of interatomic vectors in the projection of the crystal structure. If a crystal has n atoms per unit cell, its two-dimensional Patterson diagram contains n^2 peaks. Of this total, n peaks correspond with the several vectors from each of the atoms to itself; these n peaks all appear coalesced into one composite peak at the origin. The n atoms can be listed in pairs in $n(n-1)$ different ways, and to each of these there corresponds an interatomic vector (for each two atoms A and B there are two vectors, $\overrightarrow{AB}$ and $\overrightarrow{BA}$). The Patterson diagram, therefore, contains $n(n-1)$ non-origin peaks. The fact that this

diagram contains so many peaks for only n atoms per unit cell complicates its interpretation, and this limits its usefulness to cases where n is small.

3. Properties of the Difference Diagram.—Consider two crystals having the same structures but having a different species of atom occupying the same position in the two crystals, for example, the compounds $MABCD$ and $NABCD$, where M and N occupy the same positions in the two structures. For the sake of simplicity in explanation, suppose that the cell contains only one formula weight. Then the Patterson diagram of $MABCD$ contains peaks corresponding with the ends of the vectors representing the pairs $MA, MB, MC, MD; AB, AC, AD; BC, BD; CD;$ plus peaks corresponding to the reverse directions; plus peaks at the origin representing the distances between atom pairs MM, AA, BB, CC, DD. In the same way, the Patterson diagram of $NABCD$ contains peaks corresponding with the ends of vectors representing the pairs $NA, NB, NC, ND; AB, AC, AD; BC, BD; CD;$ plus peaks corresponding to the reverse vectors; plus peaks at the origin representing the distances between atoms pairs NN, AA, BB, CC, DD. If the heights of these two Patterson diagrams are subtracted at each point, then the peaks common to the two diagrams vanish, leaving only peaks corresponding to the ends of the vectors $(M - N)A$, $(M - N)B$, $(M - N)C$, $(M - N)D$, plus peaks corresponding to the reverse vectors, plus a peak $(M - N)$ $(M - N)$ at the origin. This is a rather remarkable result, for it means that the difference diagram for a cell containing n atoms has only n peaks (of which $n - 1$ are non-origin peaks) plus the $n - 1$ centrosymmetrical peaks corresponding to reverse vectors.

If the projection of the crystal structure of $MABCD$ contains only inversion centers and if M occupies one of these, then the peaks of the difference diagram occupy the same positions as atoms of the crystal structure, i. e., the difference diagram is an undistorted picture of the centers of the atoms in the crystal structure. If the crystal structure contains no symmetry, then the peaks of the difference diagram are the same as the positions of the atoms in the structure plus the positions of the atoms in the centrosymmetrical replica of the structure.

More generally, the difference diagram is related to the projected crystal structure in the following way: To derive the peaks of the difference diagram from the plane pattern, take the several symmetrically equivalent atoms $(M - N)$ and their environs from their positions in their plane group and place these clusters at the center of the point group isomorphous with the plane group in such a way that clusters retain their mutual orientations and so that all atoms $(M - N)$ fall at the origin of the point group. If the latter contains no inversion center, this must be introduced. The resulting atom positions are the same as the peaks in the difference diagram.

To interpret a difference diagram, the point group and plane group of the projected pattern must be known, and the problem resolves itself into one

of finding positions for the atoms on the plane group, knowing the corresponding positions on the point group. The chief obstacle to this is that the point group diagram is a composite picture of the clusters surrounding the $(M - N)$ atoms in their correct position in the plane group, and question arises as to which set of atoms in the point group is this cluster. Given the several atoms of the composite cluster, there are, in general, several ways of combining them to give clusters about $(M - N)$ in the plane group. One of these is the correct cluster, but all possibilities are consistent with the original data.

In general, then, it is less easy to pass from the difference diagram to the plane pattern projection of a crystal. Nevertheless, this problem is much easier than deriving the projected crystal pattern from the Patterson diagram, because for a unit cell containing n atoms, there are $n(n - 1)$ peaks in the Patterson diagram, but only $2(n - 1)$ peaks, at most, in the difference diagram. In many cases, the additional peaks in the Patterson diagram may be of aid in deciding between possible projected crystal structures predicted by the difference diagram, especially since the additional peaks are known to be of the type AB, AC, AD, BC, etc.

These principles may be extended to three-dimensional difference diagrams. Furthermore, a difference diagram made by subtracting the heights of Harker[4] diagrams has the interesting property of showing only peaks corresponding to interatomic distances of symmetry-related substitution atoms $(M - N)$. This diagram is, therefore, of aid in locating the substitution atoms $(M - N)$, about which the remainder of the atoms are clustered.

4. Practical Derivation of Difference Diagrams.—The difference diagram has been described as a diagram whose height at any given point is equal to the difference at the same point of the two Patterson diagrams for two crystals $MABCD$ and $NABCD$, i. e.,

$$\Delta_{x,\,y;\,M-N} = A_{x,\,y;\,M} - A_{x,\,y;\,N} \tag{2}$$

$$= \sum\sum \left| F_{hk0;\,MABCD} \right|^2 e^{2\pi i(hx + ky)} - \sum\sum \left| F_{hk0;\,NABCD} \right|^2 e^{2\pi i(hx + ky)}. \tag{3}$$

By expansion, this proves to be

$$\Delta_{x,\,y;\,M-N} = \sum\sum (|F_{MABCD}|^2 - |F_{NABCD}|^2)_{hk0}\, e^{2\pi i(hx + ky)}. \tag{4}$$

It follows from this that, if one makes x-ray diffraction records for two crystals $MABCD$ and $NABCD$, the value required for each term in the parentheses of (4) is simply the difference between measured F^2 values for the same reflection for the two crystals. This makes it easy to carry out the synthesis directly without necessarily plotting first the Patterson diagrams of the two crystals. It also makes it possible to carry out the synthesis

optically[5,6] by simply making each hole in the appropriate grating with an area proportional to the difference of the F^2's of the corresponding reflections of the two crystals. Optically negative holes[5,6] may be necessary in certain instances.

As a practical matter, the difference diagram need not be constructed from data obtained from two crystals having the substitute atom completely different. The two crystals may be somewhat differing members of a solid solution series in which the character of the solid solution is that of a single substitution. The origin is then the substituted atom.

5. Application to Determining the Character of Solid Solutions.—Solid solutions are structurally one of three types: Addition, subtraction or substitution or a combination of these types. Each of these may be formally regarded as substitution of N for M, where M and N are either real atoms or where one is a void. If a difference diagram is constructed using two members of a solid solution series as experimental crystals, the result is a difference diagram referred to the position of the substituted atom in the solid solution. From an analysis of this diagram, therefore, the environment of the locus of solid solution can be determined; and, if the general structural scheme is known, the type of solid solution structure can be determined.

6. The Difference Diagrams for More Than One Substitution.—In the preceding sections, properties of the difference diagram for the case of a single substitution have been discussed. If there are more substitutions, the number of peaks per unit cell are given by the middle column of the following table:

	1 SUBSTITUTION PER CELL OF n ATOMS	s SUBSTITUTIONS PER CELL OF n ATOMS	n SUBSTITUTIONS PER CELL OF n ATOMS
Total number of peaks per cell, including the several unresolved at the origin	$2n - 1$	$s(2n - s)$	n^2
Number of non-origin peaks	$2(n - 1)$	$s(2n - s - 1)$	$n(n - 1)$
Number of centrosymmetrical pairs of non-origin peaks	$n - 1$	$\frac{s}{2}(2n - s - 1)$	$\frac{n}{2}(n - 1)$

If there are n substitutions, the number of peaks per cell becomes identical with the number in a Patterson diagram.

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SYNTHESIS OF VITAMINS BY INTESTINAL BACTERIA

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Recent studies on growth factors in microörganisms have contributed much to our general knowledge of nutrition and have provided a basis for devising quantitative microbiological assays for vitamins occurring in both plant and animal materials. Deficiencies of special growth factors for yeasts, molds and bacteria have received much attention recently, but the synthesis of vitamins by these non-green plants, of equally great significance, has not been extensively investigated.

The extent to which bacteria living normally in the alimentary tract of an animal may synthesize growth factors and contribute directly to the vitamin requirements of the animal constitutes a problem of some importance. The present paper represents an attempt to determine the approximate amounts of certain B vitamins produced by species of intestinal bacteria grown as pure cultures in a chemically defined medium.

Synthesis of vitamins has already been reported for a considerable number of bacteria. Snell and Strong[1] demonstrated synthesis of riboflavin by lactic acid bacteria. Silverman and Werkman[2] showed that certain propionic acid bacteria make thiamine or its intermediates. Some strains of dysentery bacilli[3] are able to form thiamine, and also Coenzyme I or II, riboflavin and perhaps biotin. It has been reported that a strain of diphtherial organisms[4] can make thiamine, Coenzyme I or II and riboflavin.

The evidence obtained by several investigators indicates that bacteria normally living in the rumina of herbivores, such as sheep and cattle, produce considerable quantities of vitamins. Thus it has been found that the common *Bacillus vulgatus* living in the intestines of herbivores is capable of synthesizing thiamine.[5] Recently it has been shown[6] that considerable amounts of riboflavin, pyridoxine and the antihemorrhagic vitamin are formed in the rumina of sheep and cows fed on diets low in these vitamins, and the source of the vitamins is assumed to be commensal microörganisms. Almquist, *et al.*,[7] demonstrated that common bacteria, such as *Bacillus subtilis* and *Escherichia coli*, can synthesize vitamin K. The phenomenon of refection which gives protection against certain vitamin deficiencies in labo-

ratory animals indicates that the normal intestinal flora of mammals may synthesize appreciable amounts of vitamins.

The intestinal bacteria employed in the present study were kindly supplied by Dr. George Valley, Department of Bacteriology, Yale University. The organisms, *Escherichia coli*, *Proteus vulgaris*, *Bacterium aerogenes*, *Alcaligenes fecalis*, *Bacillus mesentericus* and *B. vulgatus*, were grown as stock cultures on Difco nutrient agar. A special liquid medium prepared for the studies of growth factor production was made up as follows: K_2HPO_4, 1.0 gm.; $MgSO_4 \cdot 7H_2O$, 0.1 gm.; NaCl, 5.0 gm.; $CaCl_2$, 0.005 gm.; glucose, 10.0 gm.; recrystallized asparagine, 2.6 gm.; l-tryptophane, 0.1 gm.; l-cystine, 0.05 gm.; redistilled water, 1 liter. Small measured amounts of the following trace elements were added: Fe, Mn, B, Zn, Cu and Mo. The medium was adjusted to pH 6.8 and sterilized by autoclaving at 15 lb. for 15 minutes. The inoculum was prepared by transferring a small amount of the organisms growing on agar to 5 ml. of physiological salt solution in test tubes. With a sterile pipette 0.1 ml. of the saline suspension was inoculated into 20 ml. of sterile culture liquid contained in small Erlenmeyer flasks. One set of inoculated flasks was kept as a control with growth inhibited in a room at −2°C., while the other was maintained in an incubator at +36°C. The period of growth was 48 hours unless stated otherwise.

TABLE 1

VITAMIN CONTENT OF BACTERIAL CULTURES GROWN FOR 48 HOURS AT 36°C. AND INOCULATED MEDIUM KEPT AT −2°C.

SPECIES OF BACTERIA	BIOTIN		RIBOFLAVIN		THIAMINE		NICOTINIC ACID	
	MILLI-GAMMA PER ML. OF MEDIUM	MILLI-GAMMA PER ML. OF CULTURE	GAMMA PER ML. OF MEDIUM	GAMMA PER ML. OF CULTURE	GAMMA PER ML. OF MEDIUM	GAMMA PER ML. OF CULTURE	GAMMA PER ML. OF MEDIUM	GAMMA PER ML. OF CULTURE
Escherichia coli	0	1.050	0	0.048	0.023	0.075	0.004	0.028
Proteus vulgaris	0	2.385	0.001	0.044	0.023	0.104	.....	Required
Bacterium aerogenes	0.015	2.370	0.004	0.140	0.005	0.148	0.005	0.300
Alkaligenes fecalis	0.023	0.446	0	0.067	0.033	0.146	0	0.066
Bacillus mesentericus	.....	Required	0	0.023	0.015	0.103	0	0.339
Bacillus vulgatus	0.028	1.365	0	0.136	0.031	0.150	0	1.181

Proteus vulgaris appears unable to synthesize nicotinic acid and *B. mesentericus* is deficient in biotin. It was found necessary, therefore, to add the deficient vitamin to the basal medium for these species in order to obtain growth so that tests could be made for the other vitamins which might be synthesized.

The methods used in assaying for riboflavin, biotin and nicotinic acid involved the use of *Lactobacillus casei* ϵ, *Saccharomyces cerevisiae F. B.* and *Lactobacillus arabinosus* as indicators in microbiological tests described by Williams and others.[8] Thiamine activity was tested by the *Phycomyces* assay method.[9] Growth of the bacteria to be tested was measured with a turbidimeter, and the fresh weight of the cells in each culture was calculated by reference to standard fresh weight turbidity graphs prepared for all the species studied. At the end of the growth period, all cultures were acidified with sufficient concentrated H_2SO_4 to make the liquid approximately 1 *N*. The acidified cultures were autoclaved at 15 lb. pressure for 30 minutes to effect hydrolysis of the cells. The samples were cooled, brought to pH 5.0 with NaOH and diluted to standard volume. The amounts of the solutions to be used in making the tests were determined by preliminary trials, and

TABLE 2

SYNTHESIZED VITAMIN RESIDUES IN CULTURES GROWN FOR 48 HOURS AT 36°C. VALUES EXPRESSED AS GAMMA PER GRAM OF FRESH CELLS

SPECIES	BIOTIN	RIBOFLAVIN	THIAMINE	NICOTINIC ACID
E. coli	2.3	106	115	62
P. vulgaris	3.2	57	95	None ?
B. aerogenes	1.1	41	43	89
A. fecalis	0.5	78	132	77
B. mesentericus	None ?	14	53	204
B. vulgatus	0.8	82	72	709

appropriate aliquots were employed at two concentration levels for each vitamin assay so that growth of the indicator organism would fall within a suitable range of response.

Some of the results obtained with vitamin assays performed on six species of bacteria are shown in table 1. Each value in the table represents the average of four or six determinations. The whole series of assays were repeated at different times on different cultures. In actual practice the test organisms gave satisfactorily consistent responses both to varied amounts of synthetic growth factors and to additions of bacterial extracts.

As indicated in table 1, the cultures which had grown at 36°C. for 48 hours showed a higher content of the four vitamins per ml. of fluid than did the inoculated medium in which growth was inhibited by low temperature. The results are taken to mean that under the conditions of the experiment these species of bacteria synthesize B vitamins in greater amounts than are used in their metabolism, and the residues accumulate in the cultures. The biotin, nicotinic acid, riboflavin and thiamine accumulated by the growing organisms were calculated as gamma per gram of fresh bacterial cells. These data are shown in table 2. The quantity of biotin was much lower

than the other growth factors found in the cultures. It is generally known, of course, that biotin exhibits biological activity in exceedingly small amounts. What the influence of different cultural conditions might be upon production of vitamins by bacteria would be worth further study.

An important question is how much of the total growth factors produced may be liberated from the cells into their environment. The few tests which have been made on filtrates and whole cultures of *E. coli* and *B. aerogenes* indicate that from 1 to 15% of the total biotin and nicotinic acid present in 48-hour cultures may occur outside the cells, while somewhat larger portions of the riboflavin and thiamine appear to be leached from the bacteria. In cultures of *B. aerogenes* which had grown for 111 hours, about 30% of the biotin, thiamine and riboflavin and 40% of the nicotinic acid produced by the bacteria were found in the filtrate. Age of the cultures and other factors appear to be important in determining the distribution of these water-soluble vitamins between the microörganisms and the medium in which they live.

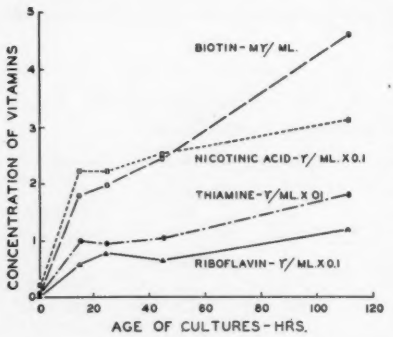

FIGURE 1

Concentration of vitamins in cultures of *B. aerogenes* grown in a chemically defined medium for different periods of time. Maximum values per ml. of culture suspension were as follows: biotin, 4.6 mγ; nicotinic acid, 0.31 γ; thiamine, 0.18 γ; riboflavin, 0.12 γ.

A study was made concerning the vitamins occurring in cultures of *B. aerogenes* at different periods of time up to 111 hours after inoculation of the basal medium. The results of this study are shown in the accompanying figure 1. It appears that the bacteria synthesized comparatively large amounts of vitamins during the early stages of growth. At $14^1/_2$ hours the thiamine, nicotinic acid and riboflavin content of the cultures attained values almost as great as those reached subsequently up to 48 hours. The biotin content increased continuously throughout the entire period. From the standpoint of utilization of growth factors by the bacteria, it is significant that synthesis of the vitamins occurred early in the period of growth. Information of this kind should be valuable also in any attempt to estimate possible uses which may be made of the vitamins synthesized by bacteria.

In recent times the synthetic powers of microörganisms have been steadily assuming greater significance in relation to human welfare, as, for example, in the employment of bacteria and molds in the dairy and chemical industries and the use of yeast for the sake of its B vitamins as a supple-

ment in the diet of man. The extent to which microörganisms living normally in the alimentary tract of an animal may synthesize vitamins and contribute directly to the vitamin requirements of that animal constitutes a problem which has not yet received adequate attention. Demonstration of the synthesis of vitamins by intestinal bacteria, as presented in this report, should have considerable significance in connection with further investigations on the nutritional relationships existing among microörganisms, animals and man.

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THEORY OF THE EFFECTS OF LIGHT INTENSITY AND DURATION IN DETERMINING VISUAL RESPONSES

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In this paper are presented some applications to the phenomena of brightness discrimination and absolute threshold measurements of a theory based partly on the familiar differential equation proposed by Hecht[2] to account for some of the phenomena of the sensory process. A generalized form of this equation is

$$\frac{dx}{dt} = \sum_{i=1}^{N} \left[k_{1i} I \left(a_0 - x \right)^{m_i} - k_{2i} x^{n_i} \right],$$

where, for vision, I represents the intensity of the exciting light, x the concentration of photoproducts broken down from the original concentration a_0 of the light-sensitive substance, t represents time, m_i and n_i are integral exponents indicating the order of the reactions and k_{1i} and k_{2i} are dimen-

sional constants characteristic of the individual eye. For simplicity in the practical applications we consider here only the bimolecular form of the equation, for which $N = 1$, $m_1 = n_1 = 2$, and $k_{11} = k_1$, $k_{21} = k_2$. It is to be noted, however, that the theory can be developed in an analogous manner for the more general equation in which both bimolecular and monomolecular reactions are included.

We assume that the threshold responses with which we are dealing occur when the concentration x changes by a constant amount Δx. To calculate this quantity it is necessary to find the solution $x(t)$, of the differential equation, appropriate to the given experimental conditions. In order to carry this out, we further assume that, whatever the variation of I with t, the solution, $x(t)$, is to be a continuous function of t. In case I is constant in some interval $t_1 < t < t_2$, then the general solution in this interval is

$$x = -\frac{1}{a}\frac{du}{dt}\frac{1}{u} = -\frac{1}{a}\left(\frac{u_1U_1e^{u_1t} + u_2U_2e^{u_2t}}{U_1e^{u_1t} + U_2e^{u_2t}}\right), \qquad (1)$$

where u_1, u_2 and a are constants involving I and the fundamental constants appearing in the differential equation. U_1 and U_2 are arbitrary constants. Our investigations are restricted to those conditions where coupling effects between adjacent regions of the retina, such as inhibition and facilitation, may be neglected. Further we require that the angular areas of the retina excited by the light are small enough to be essentially homogeneous and are large enough that the response is an average of a large number of single cell (or nerve) responses.

The method used to derive the results presented here is applicable to a large class of problems. The experimental conditions defining this class are those in which the intensity I is constant in each of a finite or "infinite" number of time intervals, $t_{i-1} < t < t_i$. That is, $I = I_i$ = constant in the ith interval.

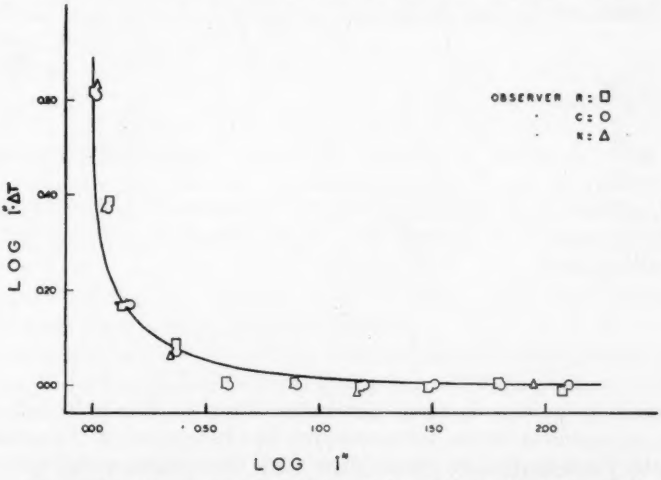
The simplest case to be treated here is that of absolute threshold with a dark-adapted eye. After this we consider the more general condition in which there is a change, at the instant $t = 0$, from the prevailing intensity I' to the intensity I''. This case includes, as special cases, the preceding one and the final one to be considered. In the last case we derive the minimum time, Δt, for the prevailing brightness, of intensity I', to be obliterated when a "dark" flash, $I'' = 0$, is detected.

I. Absolute Threshold.—The experimental conditions require that, for a long while previous to the time $t = 0$, $I = I' = 0$. At the instant $t = 0$, I becomes equal to I'' and remains constant until the instant Δt, when I again becomes equal to zero. Δt is the minimum exposure time required for the detection of the flash. The continuity condition requires that at $t = 0$, $u[= U_0e^{-\int a x dt}]$ and $(1/a)(du/dt)$ are continuous.

Applying these conditions, we find after some algebraic manipulation an expression for Δx in terms of I'' and Δt. Since we are considering only bimolecular reactions, solution for Δt of this expression yields

$$\Delta t = \frac{B}{2\sqrt{I''}} \log \frac{G\sqrt{I''} + 1}{G\sqrt{I''} - 1}, \qquad (2)$$

where B represents the constant $1/(a_0\sqrt{k_1k_2})$ and G the constant $[(a_0/\Delta x) - 1]\sqrt{k_1/k_2}$. If we suppose $\sqrt{I''} = (1/G)$, then Δt becomes infinite; or if we suppose $G\sqrt{I''} >> 1$, then $I''\Delta t \to B/G$. Hence we are led to the empirical laws of $I = C$ and $It = C$ for long and short durations, respectively.



The relation between log $I''\Delta t$ and log I'' for absolute threshold, using a twenty-minute foveal area. The curve is described by equation (2). These data were obtained from Karn.[3]

By a simple transformation of equation (2) it may be seen that the constants B and B/G can be read directly from a plot of log $I''\Delta t$ vs. log I'', obtained from the data of any given observer. It is seen that the curve for an observer is translated parallel to itself when the asymptotic constants for that observer are changed. Since the shape of the theoretical curve does not depend on any of these constants characterizing the individual, the curves for all observers should coincide when the experimental data are so translated by the appropriate addition of these constants. The congruence is obvious in the accompanying figure, showing Karn's data for three observers.[3] Karn obtained these data using a circular area subtending a visual angle of twenty minutes in diameter. The agreement with the curve derived from equation (2) is satisfying. Furthermore, by extra-

polating from Karn's experimental data, we may expect that the agreement with a somewhat larger area, e.g., sixty minutes, would be excellent.

II. Brightness and Darkness Discrimination.—The measurement of absolute thresholds is a special instance of the general case where some intensity I' has prevailed over the particular retinal area for a long time antecedent to the instant $t = 0$. In the preceding case, absolute threshold, this prevailing intensity is zero. If a flash ΔI is added (or subtracted) to I' at the instant $t = 0$ to render a new intensity $I'' = I' + \Delta I$ for a duration Δt, we find

$$\Delta t = \frac{B}{2\sqrt{I''}} \log \left\{ \frac{1 + H \dfrac{\left(1 + R\sqrt{I'}\right)\left(1 - R\sqrt{I''}\right)}{\sqrt{I'} + \sqrt{I''}}}{1 + H \dfrac{\left(1 + R\sqrt{I'}\right)\left(1 + R\sqrt{I''}\right)}{\sqrt{I'} - \sqrt{I''}}} \right\}, \quad (3)$$

where $H = \left|\Delta x/a_0\right| \sqrt{k_2/k_1}$, if $I'' > I'$, and $H = -\left|\Delta x/a_0\right| \sqrt{k_2/k_1} = -N$, if $I'' < I'$, and $R = \sqrt{k_1/k_2}$. When $I' = 0$, this equation reduces at once to (2).

III. "Inverse" Absolute Threshold.—If now, in equation (3), we suppose $I'' \to 0$ throughout the interval $0 < t < \Delta t$, then $H = -N < 0$, and we have in the limit

$$\Delta t = \frac{BN}{\sqrt{I'}} \frac{\left(1 + R\sqrt{I'}\right)^2}{\sqrt{I'}\left(1 - NR\right) - N}. \quad (4)$$

As $I' \to \infty$, $\Delta t \to R^2(B/G)$, and as $\sqrt{I'} \to N/(1 - NR) = 1/G$, $\Delta t \to \infty$. Equation (4) is the theoretical relation describing the data to be expected from measurement of the minimum time of obliteration of a prevailing intensity for detection of a "dark" flash. We have chosen to call this time "inverse" absolute threshold. It is hoped that experimental tests of this relation will soon be forthcoming.

The constants G, B/G and R may be determined empirically from data obtained in experiments performed under the conditions described in I and III. The value of N (or H), for the particular observer characterized by these constants, follows immediately from the relation $H = 1/(G + R)$. Data from brightness discrimination experiments for this observer may then be examined to determine whether they are adequately described by the theoretical curve computed from equation (3), using these values of the constants. If the comparison is satisfactory, it may be considered as an experimental check of the values of the constants and of the theory outlined here.

[1] Bartlett, N. R., and Hudson, G. E. (manuscript in preparation).

[2] Hecht, S., *Physiol. Rev.*, **17**, 239–289 (1937).

[3] Karn, H. W., *Jour. Gen. Psychol.*, **14**, 360–369 (1936).

STATIONARY POINTS OF TRANSFORMATION GROUPS

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Let (G, S) be a continuous realization of the topological group G by topological transformations of the space S into itself. The word "realization" is to be understood in its broadest sense: The transformation group (G, S) is a homomorphic, but not necessarily isomorphic, image of G. Points of S which are fixed under all the transformations in (G, S) will be called stationary and their totality denoted by $\sigma(G, S)$. We shall examine the structure of σ.

Definition 1. A compact finite-dimensional space S will be called an (n, p)-sphere $(n \geqq 1)$ if (1) S has the same homology groups, coefficients mod p, as an n-sphere; (2) no proper subset of S satisfies (1). A $(0, p)$-sphere is a pair of points; the empty set is a $(-1, p)$-sphere. It can be shown that the dimension of a locally euclidean (n, p)-sphere is n.

Let G be a topological group, and let n, p be non-negative integers. If for every (n, p)-sphere S and realization (G, S), $\sigma(G, S)$ is an (m, p)-sphere $(-1 \leqq m \leqq n)$, we shall say that *proposition* $[G, n, p]$ *is true.* If every realization (G, E_n) (E_n = euclidean n-space) admits at least one stationary point, we say that *proposition* $[G, n]$ *is true.*

I. Proposition $[G, n, p]$ *holds for prime* p *and arbitrary* n *if* G *is a finite abelian group whose order is a power of* p.

If G is cyclic, I follows from known theorems concerning periodic transformations.[1] Starting with this, the proof of I is similar to that of

II. Proposition $[G, n, p]$ *holds for prime* p, *arbitrary* n *and connected compact abelian Lie group* G.

Proof. Let I_a or $I(a)$ denote the fixed-point set of a—that is, of the transformation $x \to a \cdot x$ $(a \in G, x \in S)$. The subgroup $G^p = \{\alpha\}$ of elements of G whose orders are powers of p is everywhere dense in G. By I, I_α is an (h, p)-sphere, $h = h(\alpha)$. Since $h = n$ would imply $I_\alpha = S$ which, if true for every α, implies $\sigma(G, S) = S$, we may suppose that for some α, h is smaller than n. Since G is abelian, $a \cdot I_\alpha = I_\alpha$ for every $a \in G$. Thus there is induced in I_α a realization (G, I_α) and evidently $\sigma(G, S) = \sigma(G, I_\alpha)$. The theorem now follows by induction on n.

III. Proposition $[G, n]$ *holds for arbitrary* n *and compact connected abelian Lie group* G.

For, on adjoining a point P_∞ to E_n, we obtain a realization (G, S) where S is an ordinary n-sphere. Since P_∞ is stationary, $\sigma(G, S)$ is non-empty, hence contains at least two points. Hence $\sigma(G, E_n)$ contains at least one point.

We now consider realizations of non-abelian groups. Whether proposition $[G, n, p]$ holds for compact connected Lie groups G, arbitrary n and some p, is at present a matter of conjecture. We answer the question in various special cases.

Definition 2. We shall call (G, S) *normal* if to each element a of G we can associate an element α of order 2 such that $I_a = I_\alpha$.

Example. Suppose that G is a compact connected Lie group and A is a maximal connected abelian subgroup of G. If the induced realization (A, S) is normal then so is (G, S). This follows from the fact that two A's are transformable one into the other (Cartan, Weil[2]).

IV. Within the class of normal realizations, proposition $[G, n, 2]$ *holds for every compact Lie group* G *and* $n \geqq 0$.

Proof. Let "sphere" mean "$(m, 2)$-sphere." By normality and theorem I, every I_a is a sphere. Let $\{\alpha\}$ be the elements of order 2 in G. By induction on k, we see that intersections of k sets $I(\alpha)$—hence by normality, intersections of k sets $I(a)$—are spheres. For let $I = \bigcap_{i=1}^{k} I(\alpha_i)$. Since $\alpha_k \cdot I(\alpha_k\alpha_i) = I(\alpha_k{}^{-1}(\alpha_k\alpha_i)\alpha_k) = I(\alpha_i\alpha_k) = I(\alpha_k\alpha_i)^{-1} = I(\alpha_k\alpha_i)$, α_k induces a transformation in the set $Q = \bigcap_{i=1}^{k-1} I(\alpha_k\alpha_i)$, which is a sphere by normality and the hypothesis of induction. The fixed-point sphere is $I(\alpha_k) \cap Q$ which, one can easily see, is precisely I. The theorem now follows readily from the fact that $\sigma = \bigcap I_a$, $a \in G$.

Definition 3. The *locally euclidean case* is that in which the space under transformation is locally euclidean. If in addition the functions defining (G, S) are analytic relative to families of analytically connected local coordinate systems in G and S, we call (G, S) *analytic.*

V. In the locally euclidean case proposition $[G, 3, 2]$ *holds for every compact connected Lie group* G.

Proof. Let ξ be a point of σ. Since G is connected, orientation in the neighborhood of ξ cannot be reversed by any element a of G. Hence for α of order 2, I_α is either the $(3, 2)$-sphere S or a simple closed curve. As in the proof of IX below, we may assume that the homomorphism $G \to (G, S)$ is one-one. Hence $I_\alpha \neq S$. Let A be a maximal connected abelian subgroup of G. There is induced in the simple closed curve $I_\alpha(\alpha \in A)$ a realization (A, I_α) (cf. proof of II). If β is an element in A of any finite order, its fixed-point set cannot be empty, otherwise $\sigma(G, S) = 0$; nor can it be a pair of points, otherwise β reverses orientation in I_α. The only remaining possibility is that $I_\beta = I_\alpha$. Since the elements β are everywhere dense in A, we have $I_b = I_\alpha$ for every $b \in A - \{e\}$. Hence (A, S) is normal and hence (G, S) is normal (see example above).

VI. (Corollary). Proposition $[G, 3]$ *holds for every connected compact Lie group* G.

Suppose (G, S) is analytic. Let $n = \dim S$ at ξ, ξ a point of $\sigma(G, S)$. In the space D_n of differentials at ξ, (G, S) induces a representation (G, D_n) of

G by real linear homogeneous transformations. Let J denote fixed point sets in D_n. It can be shown that $\dim J_a = \dim I_a$ in the neighborhood of ξ.

Definition 4. An analytic (G, S) is *normal at* $\xi(\xi \in \sigma)$ if the induced (G, D_n) is normal.

VII. In the analytic case theorem IV holds if normality is replaced by normality at some point ξ *of* σ.

Let R_n denote the group of rotations in E_n.

VIII. In the analytic case, proposition $[R_n, n, 2]$ *holds for every* $n \geqq 0$.

In fact, (R_n, S_n) is normal at every point ξ of σ.

IX. In the analytic case, proposition $[G, n, 2]$ *holds for every compact connected Lie group* G *and* $n \leqq 5$.

Sketch of proof of $[G, 5, 2]$. Let G_0 be the kernel of the natural homomorphism $G \to (G, S)$ and let $H = G/G_0$. We define in a natural way an analytic realization (H, S) such that the correspondence $H \to (H, S)$ is one-one and such that $\sigma(H, S) = \sigma(G, S)$. Let ξ be a point of $\sigma(H, S)$. The representation (H, D_5) induced at ξ has a decomposition

$$(H, D_5) = (H, D_h) + (H, D_\lambda) + (H, D_\mu) + \ldots$$

where $h + \lambda + \mu + \ldots = 5$ and where $(H, D_h)$is the trivial representation of degree h, and the remaining representations are irreducible. From II, we may assume that G is non-abelian. Denoting by R_3' the covering group of R_3, an examination of the possible irreducible real representations of compact groups, of degrees $\leqq 5$ shows that the only non-trivial possibilities are, up to equivalences,[3]

1. $H = R_3'$, $(H, D_5) = (R_3', D_1) + (R_3', D_4)$
2. $H = R_3$, $(H, D_5) = (R_3, D_2) + (R_3, D_3)$
3. $H = R_4$, $(H, D_5) = (R_4, D_1) + (R_4, D_4)$
4. $H = R_3$, $(H, D_5) = (R_3, D_5)$
5. $H = R_5$, $(H, D) = (R_5, D_5)$

The representations (R_3', D_1), (R_3, D_2) and (R_4, D_1) are trivial. The remaining representations are irreducible; they exist and are unique. An examination of the weights of (R_3', D_4) shows that it is normal. Since (R_3, D_3), (R_4, D_4), (R_5, D_5) are also normal, it follows that (H, D_5) is normal in cases 1, 2, 3, 5, and IX follows from VII.

There remains only case 4; this realization is not normal. Take as generators of R_3, the infinitesimal rotations A_1, A_2, A_3 about mutually orthogonal axes in ordinary space. Let Γ_i be the 1-parameter group generated by A_i. There is a unique element α_i of order 2 in Γ_i and $\{e, \alpha_1, \alpha_2, \alpha_3\}$ is a subgroup of R_3. Let a_i denote an arbitrary element in $\Gamma_i - \{e, \alpha_i\}$. We have $\alpha_i a_j \alpha_i = a_j^{-1}$ $(i \neq j)$. Let τ be an element of order 3 such that the

transformation $a \to \tau a \tau^{-1}$ permutes the α_i cyclically. It is easy to see that such a τ exists.

In a suitable x_i-coördinate system in D_5, the infinitesimal elements corresponding to the A_i are given by[4]

$$2A_1^0 = \begin{pmatrix} 0 & 1 & 0 & 0 & 0 \\ -4 & 0 & 2 & 0 & 0 \\ 0 & -6 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -4 \\ 0 & 0 & 0 & 1 & 0 \end{pmatrix} \quad 2A_2^0 = \begin{pmatrix} 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & -4 \\ 0 & 0 & 0 & -6 & 0 \\ 4 & 0 & 2 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \end{pmatrix} \quad 2A_3^0 = \begin{pmatrix} 0 & 0 & 0 & 0 & -4 \\ 0 & 0 & 0 & -2 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 2 & 0 & 0 & 0 \\ 4 & 0 & 0 & 0 & 0 \end{pmatrix}.$$

These matrices can be obtained from the well-known representations of the unitary unimodular group in two variables; that they actually constitute a representation of R_3 can be verified by computing the commutators. Denoting fixed-point sets in D^5 by J, an examination of the characteristic vectors of the A_i^0 shows that (i) the sets $J(\alpha_i)$ are 3-dimensional; (ii) $J(a_i)$ is independent of a_i in $\Gamma_i - \{e, \alpha_i\}$ and the three sets $J(a_i)$ are distinct lines.

The fixed-point sets in S_5 are analytic loci. The sets $I(\alpha_i)$ are 3-dimensional in the neighborhood of ξ and being fixed-point sets of transformations of period 2, they are (3, 2)-spheres and locally euclidean 3-dimensional throughout. Since $\alpha_1 \cdot I(\alpha_2) = I(\alpha_1\alpha_2\alpha_1) = I(\alpha_3\alpha_1) = I(\alpha_2)$ and since the fixed-point set of the transformation that α_i thus induces in $I(\alpha_j)$, $i \neq j$, is $I(\alpha_i) \cap I(\alpha_j)$, it follows that this last set, which is 2-dimensional in the neighborhood of ξ, is a (2, 2)-sphere and locally euclidean 2-dimensional throughout, hence is homeomorphic to an ordinary sphere. The relations $I(\alpha_i) \cap I(\alpha_j) \subset I(\alpha_i\alpha_j) = I(\alpha_k)$ (i, j, k distinct) imply that the three spheres $I(\alpha_i) \cap I(\alpha_j)$ are identical. Let them be denoted by μ. The sets $I(a_i)$ are analytic (1, 2)-spheres, hence simple closed curves. Since $\alpha_1 \cdot I(a_2) = I(\alpha_1 a_2 \alpha_1) = I(a_2^{-1}) = I(a_2)$, α_1 either leaves $I(a_2)$ pointwise fixed or induces in it a transformation of period 2. It is easy to show that only the first alternative can hold; hence $I(a_i) \subset \mu$. Now by (ii), $I(a_1)$ and $I(a_2)$ intersect with distinct tangents at ξ. Since μ is a sphere, there must be at least a second point ξ' of intersection. Since ξ' is stationary under the group generated by the elements a_1 and the elements a_2, and since that group is R_3, we conclude that σ contains at least two points. Consider the element τ defined above. Evidently τ permutes the sets $I(\alpha_i)$ cyclically, as well as the sets $I(a_i)$. We conclude first that $\tau \cdot \mu = \mu$ and secondly that τ is not the identity over μ. Hence τ is of period 3 over μ and admits exactly 2 fixed points in μ. Hence σ contains at most two points in μ. But $\sigma \subset I(\alpha_1) \cap I(\alpha_2) = \mu$. Hence $\sigma = \{\xi, \xi'\}$ = a (0, 2)-sphere.

[1] Smith, P. A., "Transformations of Finite Period," *Ann. Math.*, **39**, 137–164 (1938); "Transformations of Finite Period. II," *Ann. Math.*, **40**, 690–711 (1939). See also Appendix B in Lefschetz, *Algebraic Topology* (Colloquium Publications of the Am. Math. Soc.) soon to appear.

[2] Weil, A., "Démonstration topologique d'un théorèm fondamental de Cartan," *C. R.*, **200**, 518–519 (1935).

[3] Cartan, E., "Les groupes projectifs continus réels qui ne laissent invariante aucune multiplicité plane," *Jour. de Math.*, **10**, 149–186 (1914).

[4] Explicit formulae for this rèpresentation are not really essential to the proof.

NOTE ON THE t-TEST

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For a normal universe with zero mean and standard deviation σ, the distribution of the absolute value of the mean of random samples of $n' = n + 1$ and of the estimated value $s = [\Sigma(x - m)^2/n]^{1/2}$ of σ is

$$\frac{2\sqrt{n'}}{\sqrt{2\pi}\sigma} e^{-n'm^2/2\sigma^2} dm \frac{2n^{n/2}}{2^{n/2}\Gamma(n/2)\sigma^n} e^{-ns^2/2\sigma^2} s^{n-1} ds \qquad (1)$$

with limits 0 to ∞ for both m and s. In terms of σ the test of significance for the mean m is that $T = \sqrt{n'}\, m/\sigma$ be considered a normal unit variate (so that at the level $P = 0.05$, $T = 1.96$). If the value of σ is unknown, the ratio $t = \sqrt{n'}\, m/s$ is set up, m is eliminated from (1) and an integration with respect to s leads to Student's distribution, doubled for the absolute value of t; tables for the t-test give the values of t for certain levels of significance for values of n from 1 to 30.

We propose briefly to discuss the frequency distribution for T and t obtained from (1) by eliminating s, namely,

$$\frac{4n^{n/2}}{\sqrt{2\pi}\, 2^{n/2}\Gamma(n/2)} e^{-1/2(1 + n/t^2)T^2} \frac{T^n}{t^{n+1}} dT dt \qquad (2)$$

for which the limits of both T and t are 0 and ∞. It is clear that although m and s are independent in (1), the variables T and t are not independent in (2). The frequency function vanishes when $T = 0$ and when $t = 0$, except at $T = t = 0$ where it is indeterminate, taking any positive value for the proper approach. The mode and mean of T as functions of t are[1]

$$T_{\text{mode}} = \frac{\sqrt{n}}{(1 + n/t^2)^{1/2}}, \quad T_{\text{mean}} = \frac{\sqrt{2}\Gamma(n/2 + 1)}{\Gamma(n/2 + 1/2)} \frac{1}{(1 + n/t^2)^{1/2}} \qquad (3)$$

These equations are not rectilinear. The moment of order p in T and q in t for (2) about the origin is[2]

$$\nu_{pq} = \frac{2^{p/2}n^{q/2}}{\sqrt{\pi}\Gamma(n/2)}\Gamma\left(\frac{p+q+1}{2}\right)\Gamma\left(\frac{n-q}{2}\right). \tag{4}$$

The correlation coefficient between T and t is[3]

$$r = \frac{\sqrt{1-2/\pi}}{\sqrt{\dfrac{2\Gamma^2(n/2)}{(n-2)\Gamma^2(n/2-1/2)} - \dfrac{2}{\pi}}} \text{ or } r = \sqrt{\frac{n-2}{2}}\,\frac{\Gamma(n/2-1/2)}{\Gamma(n/2)} \tag{5}$$

according as T and t are limited to positive values or are allowed to range from $-\infty$ to $+\infty$; the correlation ratio of T about its regression curve is

$$\eta = \sqrt{\frac{\dfrac{\Gamma^2(n/2+1)}{(n/2+1/2)\Gamma^2(n/2+1/2)} - \dfrac{2}{\pi}}{1-(2/\pi)}} \text{ or } \sqrt{\frac{\dfrac{\Gamma^2(n/2+1)}{(n/2+1/2)\Gamma^2(n/2+1/2)}}{1}}$$

in the two respective cases.

The relative frequency for T when t is given is

$$\frac{2(1+n/t^2)^{(n+1)/2}}{2^{(n+1)/2}\,\Gamma(n/2+1/2)}\,e^{-1/2(1+n/t^2)T^2}\,T^n dT \tag{6}$$

which is a χ^2 distribution with $\chi^2 = (1+n/t^2)T^2$ and with $n' = n+1$ degrees of freedom. Thus one may find from χ^2 tables, if sufficiently detailed, what is the chance that T exceed a specified value when t is known from observation. For example, for the smallest sample ($n = 1$, $n' = 2$), the chance that T exceed 1.96 is 0.01 for $t = 0.846$, 0.05 for $t = 1.34$, 0.10 for $t = 2.23$ and is only 0.155 for $t = \infty$, although $t = 12.71$ is significant at the 0.05 level. For $n = 2$ the tabular value $t = 4.303$ corresponding to $P = 0.05$ will ensure that $T > 1.96$ in somewhat over 20% of the samples but no value of t is large enough to ensure it in 30% of the samples. For $n = 4$ the tabular value 2.776 will ensure that $T > 1.96$ in just over 30% of the samples. For $n = 8$, the tabular value 2.306 at $P = 0.05$ will ensure that $T > 1.96$ in something like 37% of the samples but no value of t can be so high as to ensure it in 95% of the samples. As the number in the sample increases indefinitely the value of t approaches 1.96 and the percentage of the samples in which $T > 1.96$ approaches 50. For $n = 29$ (a sample of 30) the value of t which will ensure that $T > 1.96$ in 50% of the samples is 2.09, whereas the value of t corresponding to 0.05 is 2.05.

In figure 1 is represented the S-T-plane for samples of 5 with T and $S = s/\sigma$ as abscissa and ordinate. The lines $T = 1.96$, $t = 2.776$ are shown which divide the significant from the non-significant part of the plane at

$P = 0.05$; as are also the lines $S = 1.67$ and $S = 0.348$ beyond which on the upper and lower sides, respectively, the frequency function (1) has an integral value of 0.025. These four lines divide the plane into ten regions: In I–V T is not significant, in VI–X it is; in I, II, III, VI, VIII, t is not significant, in IV, V, X, IX, VII, it is; in II, IV, VIII, IX, s lies between the 2.5 and 97.5 percentiles of its distribution, in I, VI, VII, III, V, X, it lies outside those limits. The curved line in the diagram is the 0.05 contour line of the frequency function (1); for the two-dimensional normal distribution (Bravais) the contour line specified by any value of the frequency function leaves outside of it a total probability equal to that value, and in this

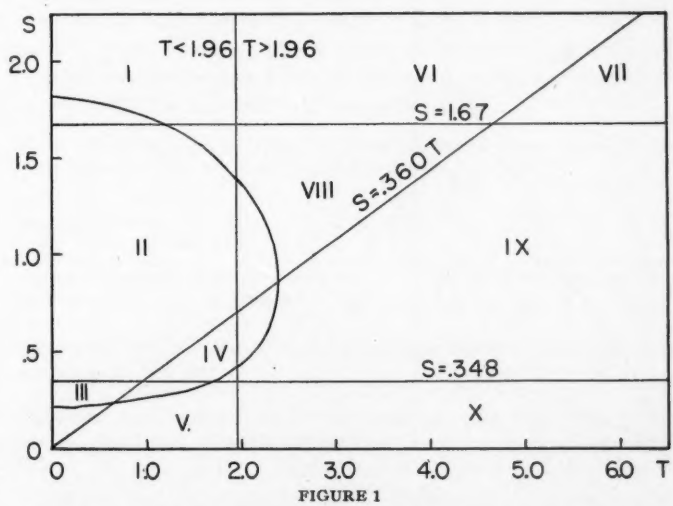

FIGURE 1

Regions of the S-T-plane connected with significance of mean, standard deviation and the ratio t, and 0.05 contour line for the probability function.

case, although (1) is not normal in s, a rough numerical integration extended over the exterior of the contour line gives 0.052. Whether one would say that the samples drawn were significantly anomalous in their values of s and m if their representative points fell outside the contour line which contained 95% of the samples when judged by the values of s and m, is a matter of definition for the individual or of convention for statisticians as a group.

In figure 2 the diagram (for samples of 5) is transferred to the T-t-plane. In the legend the probabilities that a point (sample) lie in each of the 10 regions are given. Outside the 0.05 contour line there is clearly considerable more chance of samples than 0.05, as is not unreasonable in view of the very

wide divergence of the frequency distribution from normal; a rough numerical quadrature indicates that 11% of the points lie outside the contour line.

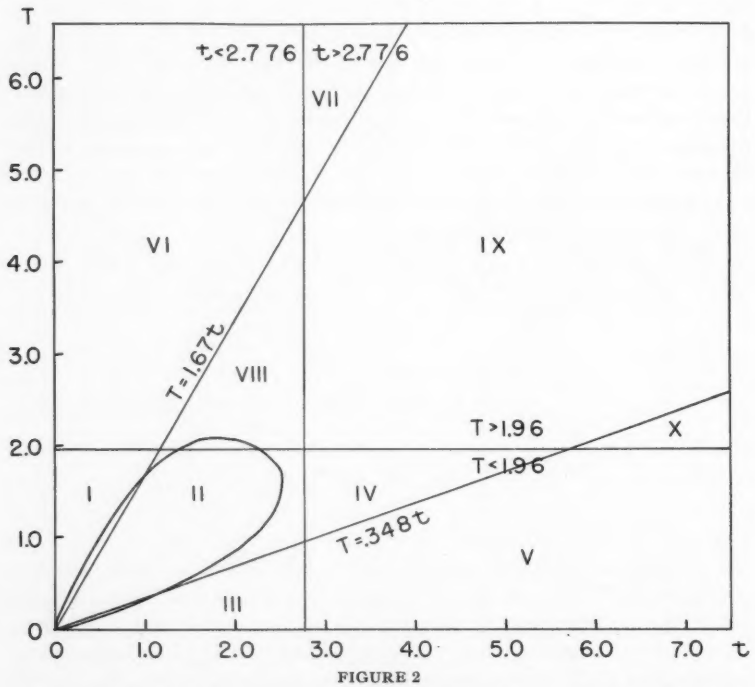

FIGURE 2

Regions of the T-t-plane connected with significance of mean, standard deviation and the ratio t, and 0.05 contour line for the probability function. The probability that (T, t) shall be in the various regions is

Region	I,	II,	III,	IV,	V,	VI,	VII,	VIII,	IX,	X
Probability	0.238,	0.8830,	0.0137,	0.0194,	0.0100,	0.0012,	0.0000,	0.0282,	0.0193,	0.0012

For samples of 3, 5, 9 (n = 2, 4, 8, respectively) the four-fold tables which correspond to the four regions separated by the line $T = 1.96$ and that for t equal to its value for $P = 0.05$, are, respectively,

	$t < 4.303$	$t > 4.303$
$T < 1.96$	0.9130	0.0370
$T > 1.96$	0.0370	0.0130

	$t < 2.776$	$t > 2.776$
$T < 1.96$	0.9206	0.0294
$T > 1.96$	0.0294	0.0206

	$t < 2.306$	$t > 2.306$
$T < 1.96$	0.9281	0.0219
$T > 1.96$	0.0219	0.0281

For these cases the correlation coefficients r and r' for the cases where T and t are both taken positive and where they are taken either positive or negative, and the corresponding correlation ratios η and η' for the regression of T on t are

	$n = 2$	$n = 4$	$n = 8$
r	0	0.756	0.899
r'	0	0.886	0.959
η	0.764	0.860	0.923
η'	0.921	0.952	0.973

Fisher has emphasized that what a fiducial argument does is to reject, or fail to reject, at a stated level of probability a hypothesis to which it is appropriate.[4] What we have here done is to discuss in some detail for the t-test the regression of the test T which one would apply if σ were known upon the value of t which is computed from the data.

[1] As t is what is known from observation it is the regression of T on t which is of interest rather than that of t on T for which the relations are linear, viz.,

$$t_{\text{mode}} = \sqrt{\frac{n}{n+1}}\,T; \qquad t_{\text{mean}} = \sqrt{\frac{n}{2}}\,\frac{(\Gamma n/2 - 1/2)}{\Gamma(n/2)}\,T.$$

[2] The value ν_{pq} is the same whether T and t are taken in absolute value or are allowed to keep their signs, provided $p + q$ is even, but if $p + q$ is odd the value of ν_{pq} is zero in the latter case.

[3] Student's distribution for t has no mean or second moment when $n = 1$ and no second moment when $n = 2$; note that (5) is imaginary for $n = 1$ and zero for $n = 2$.

[4] R. A. Fisher has pointed out very precisely the rather specific conditions under which a fiducial argument may properly be given. See his notes: *Ann. Eugenics*, **4**, 391–398 (1935) and *Ann. Math. Statis.*, **10**, 383–388 (1939).